Solid State Differentiation of Plasma Thiols using a Centrifugally Activated Mercaptobenzothiazole Disulphide Exchange Indicator

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Supporting information

Filter device preparation and operation

The centrifugal filter device (CFD) used is an Ultrapure MC 0.1 μm filter unit from Amicon Bioseparations. The CFD top compartment was packed with a layer of solid 2,2'-dithio(bis)benzothiazole (≈ 0.2 g), loaded with 500 μl of analyte solution and centrifuged at 3000 rpm for 3 minutes using Biofuge haemo centrifuge from Heraeus Instruments. The filtrate was collected in the lower compartment of the CFD. The bottom of the upper compartment consists of a porous filter membrane (0.1 μm pores size) that separates both parts of the CFD.

Analytical and clinical protocols

An analytical protocol for the CFD technique was constructed as follows: fifty micro litres from the CFD filtrate of free thiol solutions (CSH and GSH) and albumin (bovine) solution, was spiked into 2000 μl of Brittons Robinson buffer solution of pH 7. The solutions were mixed; their spectra recorded from 250 to 600 nm and analysis data taken at 312 nm. The values of absorbance at the maximum wavelength observed were plotted versus analyte concentration and the curves fitted by least-square linear regression analysis.

As far as ER test is concerned, established Ellman's Assay protocols for CSH, HCSH and GSH calibration curves were build up at 412 nm.

A clinical trial involving 2 males and 1 female control subjects, ranging from 23 to 35 years old was conducted. Blood was collected in heparinised gel permeation

vaccutainers and then centrifuged at 3000 rpm for 10 minutes. Plasma samples were tested using the CFD and ER protocols given above.

NMR spectroscopic data for MBT_thiols

In order to gain information about the species present in the filtrate of the CFD, ^{1}H NMR measurements were performed on a Bruker DRX-500 pulse Fourier transform NMR spectrometer. Typical operating conditions for routine proton measurements involved 'pulse' or flip angle of 30°, spectral frequency of 500.150 MHz, spectral width of 20.65 pp, delay time of 0.3 s, acquisition time of 3.17 s and line broadening of 0.3 Hz.. Sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) is added to all samples as secondary reference. Two CFD containing DTBT were loaded with solution of 1) free D_2O and 2) CSH in D_2O, respectively and centrifuged as described earlier. CSH is selected for this investigation due to its structure simplicity relative to GSH.

^{1}H NMR spectrum of the filtrate shows that in the case of the free D_2O, the only resonance line corresponds for the solvent (δ = 4.76 ppm), indicating that at the centrifugation rate used (3000 rpm) DTBT is insoluble and absent from the filtrate. On the other hand, when thiols were used, the chemical shifts and difference in chemical shifts recorded (see Table 1) for the set of resonance lines observed, suggest the formation of MBT_thiols mixed disulfide product solely in solution. In addition, deshielding effect is observed for the protons of the CSH molecule due to the formation of disulfide bonding with the MBT.

In addition, no peaks corresponding for the free CSH, DTBT or MBT was observed in the ^{1}H NMR spectrum of the thiols filtrate from the CFD experiment.

Protons	CSH	MBT	MBT_CSH	
	δ ppm	δ ppm	δ ppm	Δδ ppm
1	-	7.51	7.51	0.00
2	-	7.33	7.33	0.00
3	-	7.44	7.44	0.00
4	-	7.67	7.67	0.00
A	3.95	-	4.11	0.11
B	3.09	-	3.40	0.31
	3.07		3.37	0.30
C	3.03	-	3.20	0.17
	3.00		3.17	0.17

Table 1. Chemical shifts, δ ppm and difference in chemical shifts, Δδ ppm for protons of CSH, MBT and MBT_CSH in D_2O at 298 K. See structures in Fig. 1 for protons labelling.

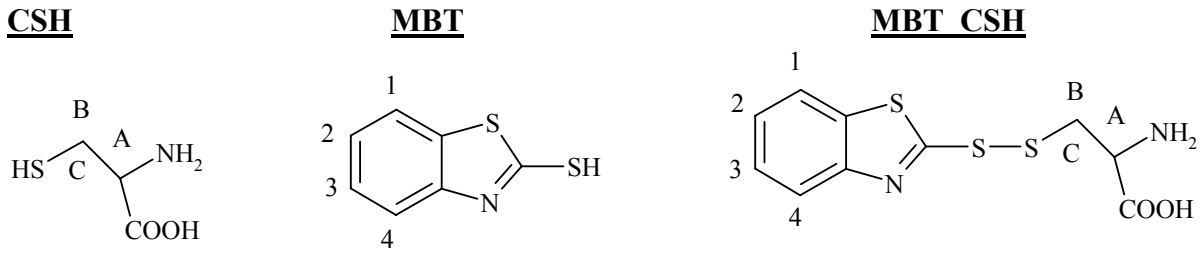

Fig. 1. Protons labelled structures of CSH, MBT and MBT_CSH, respectively.